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Stress up-regulates neuronal expression of the herpes simplex virus type 2 large subunit of ribonucleotide reductase (R1; ICP10) by activating activator protein 1

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Herpes simplex virus type 2 (HSV-2) genes expressed in neuronal cells in response to stress stimuli that trigger latency reactivation are largely unknown. Using a chloramphenicol acetyltransferase (CAT) reporter assay we found that stress caused a significant ($P < .001$) increase in ICP10 expression in neuronal cells. Up-regulation correlated with activator protein (AP)-1 activation, notably c-Jun and c-Fos that bind cognate elements in the ICP10 promoter. It was blocked by mutation of the AP-1 motifs in the ICP10 promoter. ICP10 expression protected neuronal cells from stress-induced apoptosis. The data suggest that ICP10 may contribute to HSV-2 reactivation by increasing neuronal survival. *Journal of NeuroVirology* (2005) **11,** 329–336.

Keywords: AP-1; apoptosis; HSV-2 R1; latency; neurons; stress

Herpes simplex viruses types 1 and 2 (HSV-1 and -2) establish a life-long latent infection of sensory ganglionic neurons that is largely transcriptionally silent. Latency is characterized by periodic recurrent episodes of virus reactivation, which are triggered by stress stimuli. HSV-1 studies led to the conclusion that stress-induced transcription factors, notably activator protein (AP)-1, disrupt the latent phase transcriptional repression, thereby initiating virus replication (Valyi-Nagy *et al*, 1991). They implicated viral genes ICP0 and latency-associate transcript (LAT) in latency reactivation, the latter by virtue of its anti-apoptotic activity (Loiacano *et al*, 2003; Ahmed *et al*, 2002; Jin *et al*, 2003). However, the role of transcription factors in the reactivation of latent HSV-2 and the viral genes that respond to reactivating stimuli, are still poorly understood. Because hyperthermic stress, a common latency reactivating stimulus, up-regulates heat shock proteins (Hsp's) by inducing the heat shock transcription factor (HSF) (Morimoto, 1998), it seemed reasonable to assume that Hsp-like viral proteins are similarly up-regulated. One such protein is the large subunit of HSV-2 ribonucleotide reductase (R1, also known as ICP10). ICP10 contains independent protein kinase (PK) and R1 functions, both of which are required for virus replication in nondividing (neuronal) cells (Goldstein and Weller, 1988; Smith *et al*, 2000a). The N-terminal PK domain is homologous to a small Hsp (known as H11) (Smith *et al*, 2000b; Aurelian *et al*, 2001). It contains a degenerate crystallin motif similar to that in small Hsp family members (Chabaud *et al*, 2003), and direct alignment with anchored PK motifs reveals 32% identity (23/71 identical residues) and 59% homology (42/71 identical and functionally homologous residues) between the H11 and ICP10 PK catalytic cores (Figure 1A). The studies described in this report were designed to test the hypothesis that stress induces ICP10 expression, and determine the role of transcription factors in this process.

To examine the expression of ICP10 in the absence of other viral proteins we used an ICP10-CAT (chloramphenicol acetyltransferase) reporter

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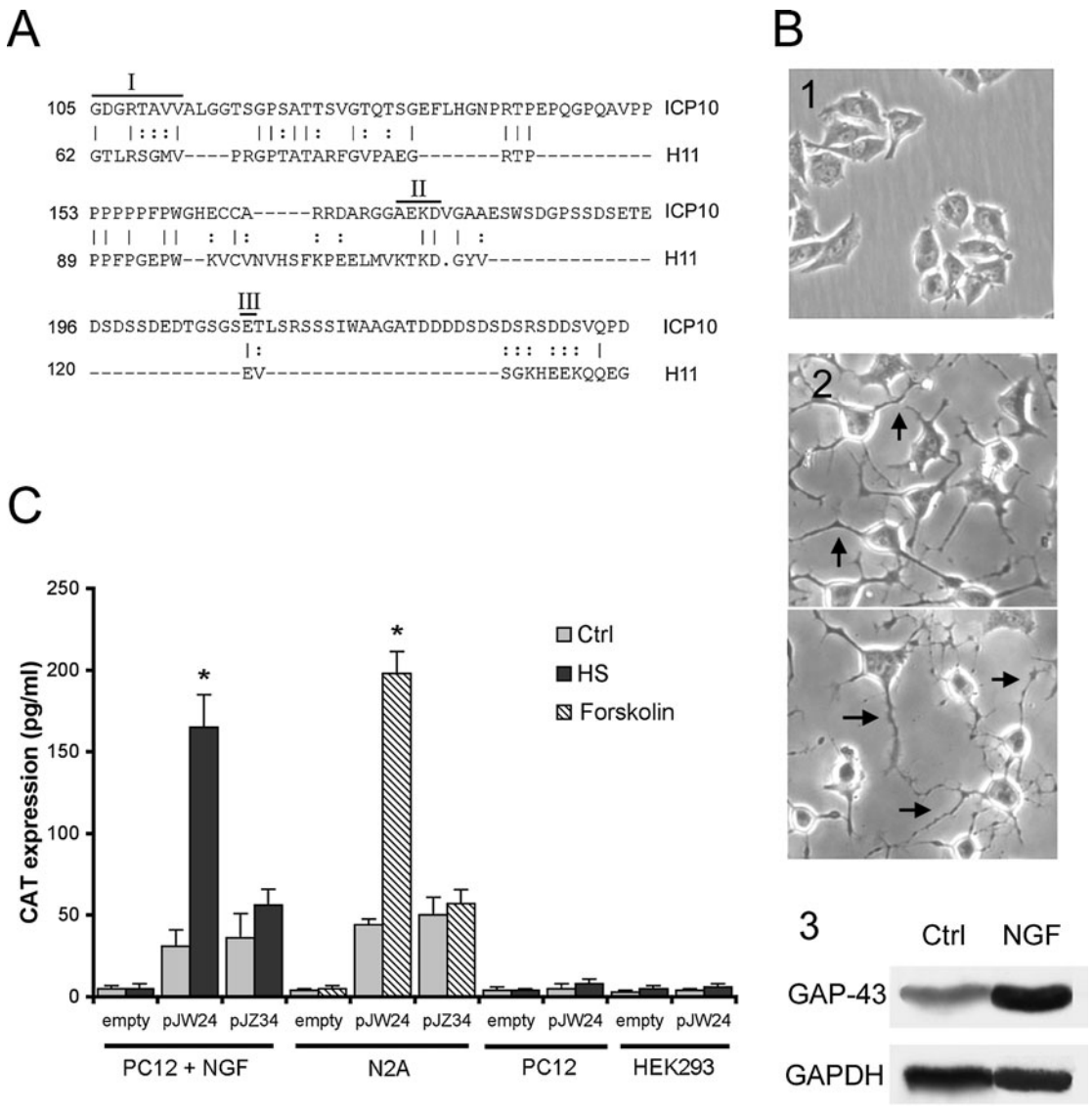

Figure 1 Heat stress triggers ICP10 promoter activation in neuronal cells. (**A**) Alignment of the catalytic cores of ICP10 (Chung *et al*, 1989) and H11 (Smith *et al*, 2000b) with anchored PK motifs (roman numerals) and using the ALIGN gene analysis software (http://www2.igh.cnrs.fr/bin/align-guess.cgi). (**B**) Proliferating PC12 cells (panel 1) exhibit characteristic rounded morphology. PC12 cells cultured with NGF exhibit neurite outgrowths (panel 2; two fields are shown, note arrows) and increased levels of the neuronal differentiation marker, GAP-43 (panel 3; NGF), as compared to proliferating PC12 cells (panel 3; Ctrl) as determined by immunoblotting with GAP-43 antibody. The levels of GAPDH (used as loading control) were similar in NGF treated and untreated cultures (panel 3). (**C**) PC12, NGF treated PC12, N2A, and HEK293 cells transfected with empty vector (empty), wild-type ICP10-CAT construct (pJW24), or the AP-1 mutant ICP10-CAT construct (pJZ34) were stressed with heat (HS) or forskolin and assayed for CAT expression. Unstressed cells (Ctrl) were studied in parallel. Results are expressed as mean CAT concentration ±SEM. $^*P < .001$ by ANOVA relative to unstressed control.

gene assay with the previously described pJW24 and pJZ34 constructs (Wymer *et al*, 1989; Zhu and Aurelian, 1997). pJW24 contains the wild-type 649-bp ICP10 promoter (−544 to +105 relative to the mRNA cap site) inserted 5′ to the CAT structural gene. Construction was in pCATB′, which lacks eukaryotic promoter sequences. pJW24-initiated transcription induces CAT mRNA production and translation, allowing for the quantification of promoter activity. In pJZ34 the two AP-1 *cis*-response elements (TGACTCA) in the ICP10 promoter were mutated (TGA**AG**CA). Mutation was by oligonucleotide-directed mutagenesis using oligonucleotides 5′-ACACCCCCCTGA**AG**CAGGAGATAGGC-3′ and 5′-CCTTAGATTGA**AG**CAGCACACG-3′ and the Muta-Gene system (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

PC-12 (rat pheochromocytoma) cells are routinely used to study neuronal cell functions. They were grown in DMEM/F12 (Dulbecco's modified Eagle medium [Invitrogen, Carlsbad, CA] and F12 nutrient mixture (Invitrogen) at 1:1 ratio, 7.75 g/L of glucose, and 7.5 mg/L sodium bicarbonate) with 10% fetal bovine serum (FBS). N2A (murine neuroblastoma)

cells were grown in Opti-MEM/DMEM (1:1 ratio) supplemented with 5% FBS, and human embryonic kidney (HEK293) cells were grown in DMEM with 10 % FBS. HEK293 cells were used as a non-neuronal cell control, based on the premise that ICP10 behaves as an Hsp and previous findings that hyperthermic stress induces Hsp expression in both PC12 and HEK293 cells (Gober *et al*, 2003; Quigney *et al*, 2003). PC12 cells were cultured (24 h, 37°C) in NB/B27 medium (neurobasal medium with 1× concentration of B27 serum-free supplement [Invitrogen])with 100 ng/ml nerve growth factor (NGF) (Invitrogen) to cause their differentiation into neurons. Prior to hyperthermic stress, the cells were cultured for an additional 24 h in serum-free DMEM/F12 medium in order to decrease the potentially confounding contribution of NGF-up–regulated transcription factors (Otten *et al*, 2000). Differentiation was evidenced by the development of neurite outgrowths (Figure 1B, panel 2) that were not seen in proliferating PC12 cells (Figure 1B, panel 1). It was confirmed by increased levels of the neuronal differentiation marker GAP43 (Das *et al*, 2004) in NGF-treated relative to untreated PC12 cells, as determined by immunoblotting (Figure 1B, panel 3).

Neuronally differentiated PC12 cells were transfected with pJW24 or empty vector (2 μg) using FuGene 6 reagent (Roche Applied Science, Indianapolis, IN) according to manufacturer's instructions. They were subjected (or not) to heat stress (43°C; 1 h) and allowed to recover at 37°C for 5 h. Cell extracts obtained at that time were assayed for CAT levels using a commercially available enzyme-linked immunosorbent assey (ELISA) kit (Roche Applied Science) and the results are expressed as pg/ml. HEK293 and undifferentiated PC12 cells were studied in parallel and served as control. CAT levels were significantly ($P < .001$) higher in heat-stressed than unstressed neuronally differentiated PC12 cells transfected with pJW24 (165 ± 20 and 31 ± 10 pg/ml, respectively), but not the empty vector (5 ± 3 and 5 ± 2 pg/ml, respectively). Heat shock did not increase the levels of CAT in pJW24-transfected PC12 cells that were undifferentiated (8 ± 3 and 5 ± 3 pg/ml for heat stressed and unstressed, respectively) nor in HEK293 cells (6 ± 2 and 4 ± 1 pg/ml for heat stressed and unstressed, respectively) (Figure 1C). The data indicate that hyperthermic stress induces ICP10 expression in neurons, but by a mechanism distinct from that involved in Hsp up-regulation.

Since peripheral and central nervous system (CNS) neurons respond to stress with AP-1 activation (Vali-Nagi *et al*, 1991, Maroni *et al*, 2003), and the ICP10 promoter has two AP-1 *cis*-response elements (Wymer *et al*, 1989; Zhu and Aurelian, 1997), we considered the possibility that stress-induced ICP10 up-regulation depends on AP-1 activation. In a first series of experiments designed to address this question, neuronally differentiated PC12 cells were transfected with pJW24 or pJZ34 (AP-1 mutant), heat shocked (43°C; 1 h), and assayed for CAT expression at 5 h after recovery (at 37°C). CAT levels in pJW24-transfected cells were significantly higher after heat stress than in its absence (165 ± 20 and 31 ± 10 pg/ml, respectively). This increase was not seen in cultures transfected with the AP-1 mutant (56 ± 10 pg/ml), suggesting that stress-induced up-regulation of ICP10 expression is AP-1 dependent (Figure 1C). In a second series of experiments to test the role of stress-induced AP-1 on ICP10 expression, we asked whether AP-1 mediated activation of the ICP10 promoter also occurs in other differentiated neurons. We took advantage of previous findings that the adenylate cyclase activator forskolin induces stress-related differentiation of N2A cells involving AP-1 activation (Mackler *et al*, 2003). This system is particularly interesting from the standpoint of ICP10 up-regulation, because forskolin induces reactivation of latent HSV in primary neurons (Colgin *et al*, 2001). N2A cells were treated with forskolin (30 μM, 48 h), transfected with pJW24 or pJZ34, and CAT expression was determined 24 h later. Forskolin caused a significant increase in CAT expression in N_eA cells transfected with pJW24 (198 ± 13 and 44 ± 4 pg/ml for forskolin treated and untreated, respectively), but not pJZ34 (57 ± 9 and 50 ± 11 pg/ml for forskolin treated and untreated, respectively). The data support the conclusion that stress induces ICP10 up-regulation in neurons, by activating AP-1. AP-1 can also up-regulate ICP10 in other cell types (Wymer *et al*, 1989; Zhu and Aurelian, 1997). However, stress-induced AP-1 activation, and thereby ICP10 up-regulation, appear to be neuron specific.

To examine the contribution of c-Fos and c-Jun towards AP-1–mediated up-regulation of ICP10, we asked whether they are activated in heat stressed neuronally differentiated PC12 cells. NGF-differentiated cells were cultured (24 h) in DMEM/F12 medium to reduce the levels of NGF-induced transcription factors, heat shocked (43°C, 1 h) and allowed to recover at 37°C for 30 min or 4 h, conditions that activate ICP10 expression (Figure 1C). Cell extracts were immunoblotted with antibodies to c-Fos (Santa Cruz Biotechnology, Santa Cruz, CA), which recognizes both the phosphorylated (P-c-Fos) and unphosphorylated forms (Burch *et al*, 2004), c-Jun, or activated (phosphorylated on Ser63) c-Jun (P-c-Jun) (Cell Signaling Technology, Beverly, MA). The levels of both the phosphorylated and unphosphorylated c-Jun and c-Fos were higher in the heat stressed than unstressed cells, indicating that they are up-regulated by heat stress (Figure 2A, lanes 1 to 3). Increased c-Fos expression/phosphorylation as late as 4 h after stress is consistent with previous reports that c-Fos is metabolically stabilized by phosphorylation (Hunter and Karin, 1992; Okazaki and Sagata, 1995). Heat shock had no effect on c-Fos and c-Jun expression and caused a minimal increase (less than two-fold) in c-Jun activation in undifferentiated PC12 cells (data not shown).

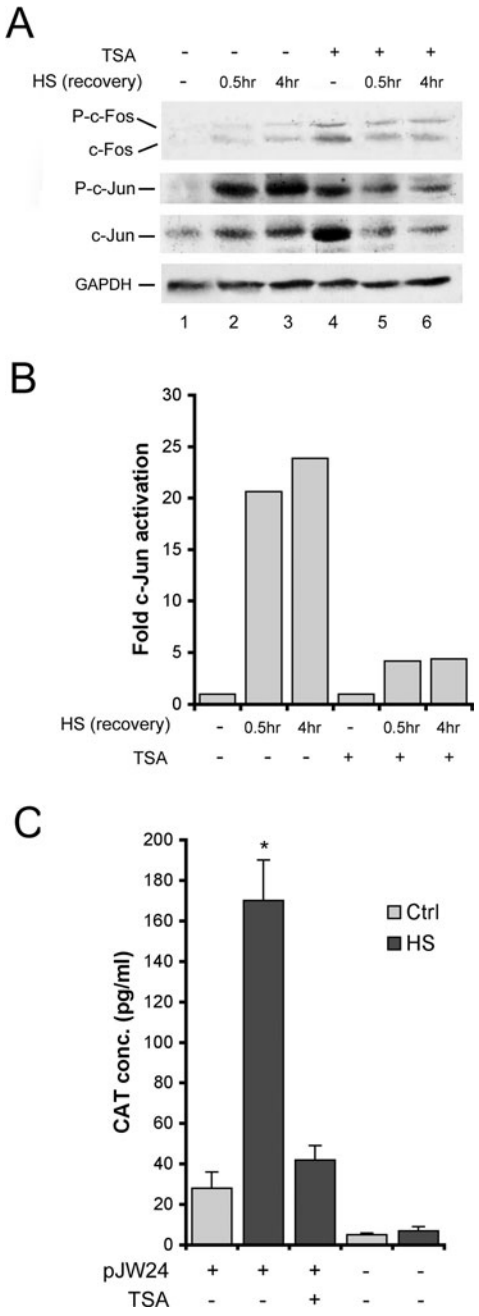

Figure 2 Heat induced ICP10 promoter activation is mediated by AP-1. (**A**) PC12 cells were cultured (24 h) in NB/B27 medium supplemented with NGF (100 ng/ml) followed by (24 h) serum-free DMEM/F12 medium with or without trichostatin A (TSA; 2 μM) and subjected (or not) to heat stress (HS). Extracts were collected at 0.5 h or 4 h of recovery (at 37°C) and immunoblotted with antibody to c-Fos (recognizes c-Fos and phophorylated c-Fos [P-c-Fos] [Burch *et al*, 2004]). Blots were stripped and reprobed with antibodies to c-Jun, P-c-Jun, and GAPDH (used as loading control). (**B**) Data from (**A**) were quantified by densitometry and expressed as P-c-Jun/c-Jun ratio normalized to the no heat stress controls (fold c-Jun activation). (**C**) Neuronally differentiated PC12 cells were transfected with pJW24 or empty vector and treated (or not) with TSA. The cultures were heat stressed (HS) or not (Ctrl) and assayed for CAT expression. Results are expressed as mean CAT concentration ± SEM. $^*P < .001$ by ANOVA relative to unstressed control.

Stress-induced Hsp expression may involve histone acetylation (Nightingale *et al*, 1998) as indicated by recent findings that Hsp70 expression is up-regulated by the class II histone deacetylase inhibitor trichostatin A (TSA) (Rizzi *et al*, 2004). Because TSA is also known to alter AP-1 activity (Sakata *et al*, 2004), we wanted to know whether its effect on c-Fos and c-Jun expression is different within the context of stress. Duplicate cultures of neuronally differentiated PC12 cells were grown in DMEM/F12 medium with or without TSA (2 μM, 24 h), heat-shocked (43°C, 1 h), and extracts obtained at 30 min and 4 h after recovery were immunoblotted with the c-Fos, c-Jun, and P-c-Jun antibodies. TSA treatment increased the levels of c-Fos and c-Jun (phosphorylated or not) in unheated cells (Figure 2A, lanes 1, 4), but this was significantly reduced by heat shock (Figure 2A, lanes 4 to 6). c-Jun levels were also significantly lower in heat-shocked cells that were treated with TSA (Figure 2A, lanes 5, 6) than in similarly heat-shocked cells that were not TSA treated (Figure 2A, lanes 2, 3). Densitometric scanning and data expression as P-c-Jun/c-Jun ratio (fold c-Jun activation) indicated that, relative to unheated cells, the levels of P-c-Jun were 20- to 24-fold higher in heat-shocked cells and only 4 to 5-fold higher in heat-shocked and TSA-treated cells (Figure 2B). TSA is known to have different modulatory effects on various genes (Eickhoff *et al*, 2000). Its negative effect on AP-1 expression/activation in heat-stressed, but not unstressed, cells may be due to increased acetylation of AP-1–specific nonhistone repressor proteins (Mulholland *et al*, 2003) and/or phosphatases.

Having seen that stress has a differential effect on c-Jun and c-Fos expression/activation when combined with TSA treatment, we wanted to know whether this correlates with ICP10 up-regulation. Neuronally differentiated PC12 cells were transfected with pJW24 and heat shocked in the presence or absence of TSA (2 μM). Hyperthermia-induced activation of the ICP10 promoter was significantly reduced in the presence, as compared to the absence, of TSA (42 ± 10 and 170 ± 18 pg/ml CAT, respectively) (Figure 2C). This is consistent with the reduced levels of c-Jun and c-Fos activation under these conditions (Figure 2B) and supports the role of AP-1 in stress-induced activation of the ICP10 promoter.

To examine whether hyperthermic stress alters the binding activity of AP-1 to its cognate response element, we used electrophoretic mobility shift assays (EMSA) with nuclear extracts from neuronally differentiated PC12 cells that were heat shocked (43°C; 1 h) and allowed to recover for 2 h at 37°C. Nuclear extracts from similarly differentiated but unstressed cells served as control. The nuclear extracts were prepared as described by Dignam *et al* (1983). Oligonucleotides containing the AP-1 elements in the ICP10 promoter (5′-CGCTTGATGACTCAGCCGGAA-3′) were labeled

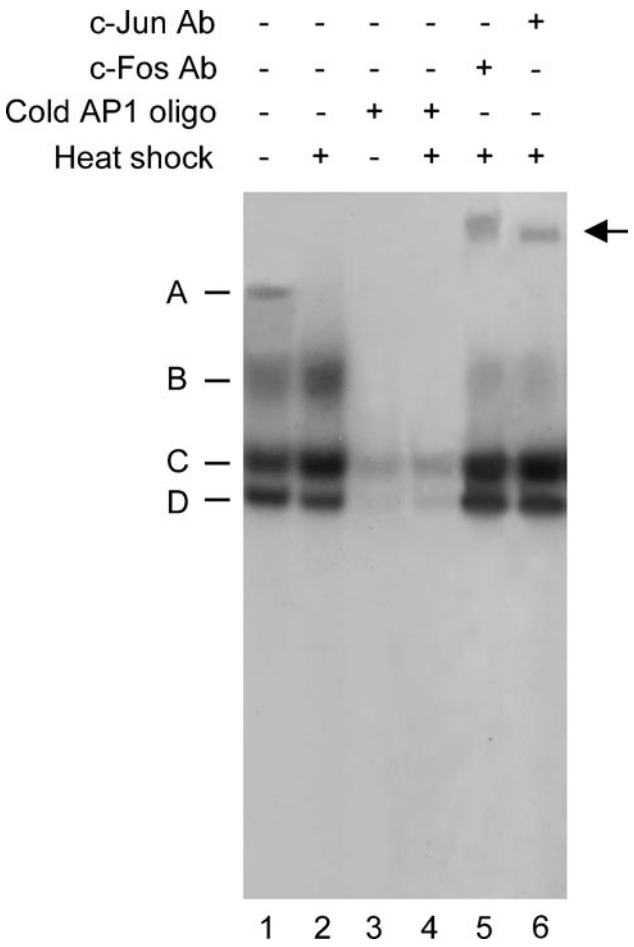

Figure 3 Stress potentiates AP-1 binding of the ICP10 promoter. Nuclear extracts from neuronally differentiated PC12 cells that were heat stressed (lanes 2, 4, 5, 6) or not (lanes 1, 3) were incubated with [γ-^{32}P]ATP-labeled oligonucleotides containing the consensus AP-1 *cis* response element in the ICP10 promoter. In some experiments, 50-fold excess cold oligonucleotides containing the consensus AP-1 binding site (lanes 3, 4), or c-Fos (lane 5), or c-Jun (lane 6) antibodies were added. Supershifted band are identified (*arrow*).

with [γ-^{32}P] ATP and incubated (20 min; 25°C) with 5 μg of nuclear extracts. They were electrophoresed (30 mA at 4°C) on a nondenaturing 5% polyacrylamide gel (38:1 acrylamide-bisacrylamide in 0.5× TBE; pre-run for 30 min). Three major complexes (B to D) and one minor complex (A) were observed with nuclear extracts from unstressed cells (Figure 3, lane 1). These complexes are AP-1 specific, because they were competed by cold AP-1 oligonucleotides (Figure 3, lanes 3, 4). Complex B was significantly increased when the binding assay was done with nuclear extracts from heat stressed cells (Figure 3, lane 2). This complex consists of c-Fos and c-Jun (c-Fos/c-Jun heterodimer), as evidenced by supershift analysis with c-Fos and c-Jun antibodies (Figure 3, lanes 5, 6). Similar results were obtained with nuclear extracts collected at 4 h after recovery (data not shown). Interestingly, complex A was not seen in binding assays with extracts from heat stressed cells (Figure 3, lane 2), suggesting that it may function to block transactivation of the ICP10 promoter. AP-1 factors in complexes C and D are probably not involved in ICP10 up regulation because these complexes were not altered by heat shock (Figure 3, lanes 1, 2). The identity of the AP-1 factors (Shaulian and Karin, 2002) involved in the formation of complexes A, C, and D is presently under investigation in our laboratory.

Because ICP10 inhibits virus-induced apoptosis in neurons (Perkins *et al*, 2003), we wanted to know whether it can also inhibit apoptosis caused by heat stress (Leoni *et al*, 2000). PC12 cells were stably

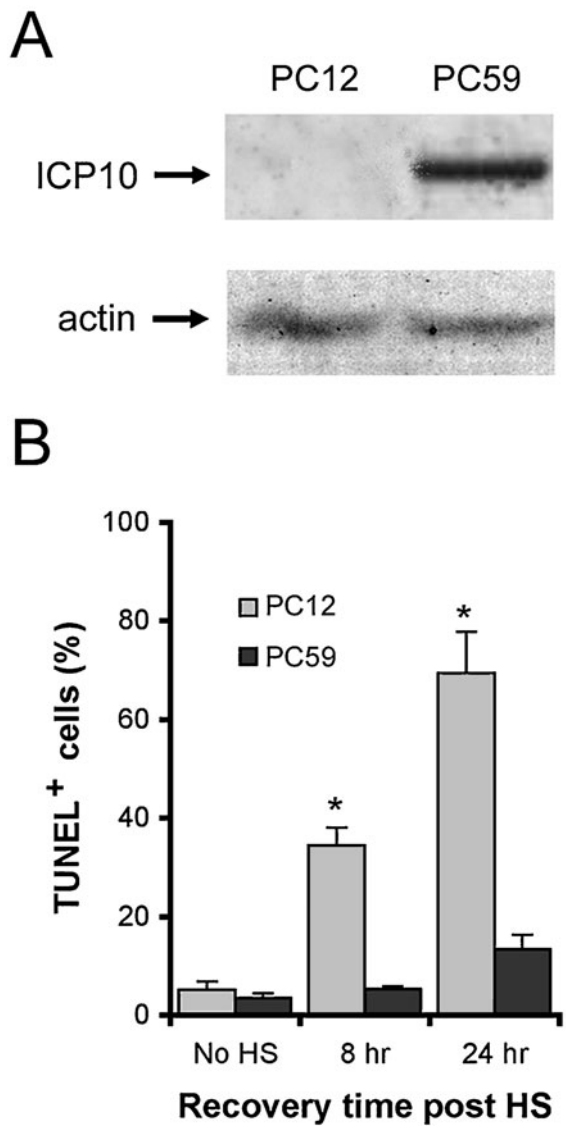

Figure 4 ICP10 expression protects from hyperthermia induced apoptosis. (**A**) Extracts from PC12 and PC59 cells were immunoblotted with ICP10 antibody. The blot was stripped and reprobed with antibody specific for actin (Santa Cruz Biotechnology). (**B**) Neuronally differentiated PC12 and PC59 cells were heat shocked (HS) (or not) and assayed for apoptosis (by TUNEL) at 8 or 24 h post recovery at 37 °C. Results are expressed as mean % TUNEL$^+$ cells ±SEM. $^*P < .001$ by ANOVA relative to similarly treated PC12 cells.

transfected with 2 μg of a previously described ICP10 expression vector (pJW17N) that contains a neomycin resistance gene. Selection was with 400 μg/ml G418 (Invitrogen) and ICP10 expression was confirmed (Figure 4A) by immunoblotting with ICP10 antibody, as previously described (Smith *et al*, 2000a). These cells (PC59) and untransfected PC12 cells were neuronally differentiated, subjected to heat shock (or not), and allowed to recover at 37°C for 8 and 24 h. They were assayed for apoptosis by TUNEL, using the *in situ* cell death detection kit (Roche Applied Science). The % TUNEL$^+$ (apoptotic) cells were significantly ($P < .001$) lower in PC59 than PC12 cells, both at 8 h (5% $\pm$ 1% and 34% $\pm$ 4%, respectively) and 24 h (13% $\pm$ 3% and 69% $\pm$ 8%, respectively) post heat shock (Figure 4B). Unstressed cultures exhibited only background levels of apoptosis (3% $\pm$ 1% and 5% $\pm$ 2% TUNEL$^+$ cell for PC59 and PC12 cells, respectively) (Figure 4B). The data indicate that ICP10 protects neurons from stress-induced apoptosis.

The data described in this report indicate that stress up-regulates ICP10 expression in neuronal cells in the absence of other viral genes. However, despite its similarity to members of the Hsp family, ICP10 is up-regulated by AP-1, rather than HSF. Up-regulation involves AP-1 *cis*-response elements in the ICP10 promoter, as evidenced by (i) the failure to induce expression from the AP-1 mutated promoter, and (ii) the results of EMSA experiments, which indicate that stress potentiates the AP-1 DNA-binding activity of nuclear extracts as late as 4 h after recovery. Although both c-Fos and c-Jun were up-regulated/activated by hyperthermic stress, we believe that c-Jun is likely to play a more prominent role in heat-induced ICP10 upregulation, because (i) the levels of P-c-Jun, were significantly higher in heat-stressed than unstressed cells, and (ii) TSA reduced the heat-induced up-regulation of ICP10 and the activation of c-Jun. Distinct AP-1 factors may be differentially involved in ICP10 up-regulation by other stressors. For example, forskolin, which also activates the ICP10 promoter, is known to increase the levels of c-Fos and Jun-B, while minimally affecting c-Jun (Vaccarino *et al*, 1993). Ongoing studies are designed to examine the contribution of distinct AP-1 family members (Shaulian and Karin, 2002) towards activation of the ICP10 promoter by various stress conditions. Notable is the identity and function of complex A, which is not seen in assays of heat stressed cells.

What is the role of ICP10 in latency reactivation? Previous studies of latency reactivation focused on HSV-1 and implicated ICP0 (Loiacono *et al*, 2003) and LAT (Ahmed *et al*, 2002; Jin *et al*, 2003), the latter by virtue of its antiapoptotic activity. HSV-1 latency reactivation was also associated with apoptosis (Hunsperger and Wilcox, 2003) and the induction by heat stress of cAMP early repressors (ICER) that down-regulate LAT (Colgin *et al*, 2001). Possibly, the involvement of ICP0 and/or LAT is specific for some, but not other, HSV-1 strains, latency models, and/or stress stimuli (reviewed in Jones, 2003). However, the role of transcription factors in the reactivation of latent HSV-2 and the viral genes that respond to reactivating stimuli are still poorly understood. LAT does not seem to be involved (Wang *et al*, 2001), a conclusion supported by the failure of the HSV-2 LAT to substitute for its HSV-1 counterpart in promoting latency reactivation (Hill *et al*, 2003). We have previously implicated ICP10 in HSV-2 latency reactivation by showing that (i) an HSV-2 mutant deleted in the PK domain of ICP10 is severely compromised for latency reactivation (Aurelian *et al*, 1999; Wachsman *et al*, 2001), and (ii) HSV-2 reactivation from explanted ganglia is inhibited by an ICP10-specific antisense oligonucleotide (Aurelian and Smith, 2000). The present findings support this interpretation, at least within the context of neuronal stressors that activate AP-1. Presumably, ICP10 contributes to virus replication after stress-induced resumption of virus transcription, because both the PK and RR functions are required for virus replication in neurons (Goldstein and Weller, 1988; Smith *et al*, 2000a). The ability of ICP10 to inhibit stress-induced apoptosis suggests that its neuroprotective PK function (Perkins *et* al, 2003) contributes to latency reactivation by increasing the number of live neurons capable of supporting virus replication.

References

Ahmed M, Lock M, Miller CG, Fraser NW (2002). Regions of the herpes simplex virus type 1 latency-associated transcript that protect cells from apoptosis in vitro and protect neuronal cells in vivo. *J Virol* **76:** 717–729.

Aurelian L, Kokuba H, Smith CC (1999). Vaccine potential of a herpes simplex virus type 2 mutant deleted in the PK domain of the large subunit of ribonucleotide reductase (ICP10). *Vaccine* **17:** 1951–1963.

Aurelian L, Smith CC (2000). Herpes simplex virus type 2 growth and latency reactivation by cocultivation are inhibited with antisense oligonucleotides complementary to the translation initiation site of the large subunit of ribonucleotide reductase (RR1). *Antisense Nucleic Acid Drug Dev* **10:** 77–85.

Aurelian L, Smith CC, Winchurch R, Kulka M, Gyotoku T, Zaccaro L, Chrest FJ, Burnett JW (2001). A novel gene expressed in human keratinocytes with long-term i*n vitro* growth potential is required for cell growth. *J Invest Dermatol* **116:** 286–295.

Burch PM, Yuan Z, Loonen A, Heintz NH (2004). An extracellular signal-regulated kinase 1- and 2-dependent program of chromatin trafficking of c-Fos and Fra-1 is required for cyclin D1 expression during cell cycle reentry. *Mol Cell Biol* **24:** 4696–4709.

Chabaud S, Lambert H, Sasseville AM-J, Lavoie H, Guilbault C, Massie B, Landry J, Langelier Y (2003). The R1 subunit of herpes simplex virus ribonucleotide reductase has chaperone-like activity similar to Hsp27. *FEBS Lett* **545:** 213–218.

Chung TD, Wymer JP, Smith CC, Kulka M, Aurelian L (1989). Protein kinase activity associated with the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). *J Virol* **63:** 3389–3398.

Colgin MA, Smith RL, Wilcox CL (2001). Inducible cyclic AMP early repressor produces reactivation of latent herpes simplex virus type 1 in neurons in vitro. *J Virol* **75:** 2912–2920.

Das KP, Freudenrich TM, Mundy WR (2004). Assessment of PC12 cell differentiation and neurite growth: a comparison of morphological and neurochemical measures. *Neurotoxicol Teratol* **26:** 397–406.

Dignam JD, Lebovitz RM, Roeder RG. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* **11:** 1475–1489.

Eickhoff B, Ruller S, Lane T, Kohler G, Stahl C, Schlaak M, van der Bosch J (2000). Trichostatin A modulates expression of p21waf1/cip1, Bcl-xL, ID1, ID2, ID3, CRAB2, GATA-2, hsp86 and TFIID/TAFII31 mRNA in human lung adenocarcinoma cells. *Biol Chem* **381:** 107–112.

Gober MD, Smith CC, Ueda K, Toretsky JA, Aurelian L (2003). Forced expression of the H11 heat shock protein can be regulated by DNA methylation and trigger apoptosis in human cells. *J Biol Chem* **278:** 37600–37609.

Goldstein DJ, Weller SK (1988). Factor(s) present in herpes simplex virus type 1-infected cells can compensate for the loss of the large subunit of the viral ribonucleotide reductase: characterization of an ICP6 deletion mutant. *Virology* **166:** 41–51.

Hill J, Patel A, Bhattacharjee P, Krause P (2003). An HSV-1 chimeric containing HSV-2 latency associated transcript (LAT) sequences has significantly reduced adrenergic reactivation in the rabbit eye model. *Curr Eye Res* **26:** 219–224.

Hunsperger EA, Wilcox CL (2003). Caspase-3-dependent reactivation of latent herpes simplex virus type 1 in sensory neuronal cultures. *J NeuroVirol* **9:** 390–398.

Hunter T, Karin M (1992). The regulation of transcription by phosphorylation. *Cell* **70:** 375–387.

Jin L, Peng W, Perng G-C, Brick DJ, Nesburn AB, Jones C, Wechsler SL (2003). Identification of herpes simplex virus type 1 latency-associated transcript sequences that both inhibit apoptosis and enhance the spontaneous reactivation phenotype. *J Virol* **77:** 6556–6561.

Jones C (2003). Herpes simplex virus type 1 and bovine herpesvirus 1 latency. *Clin Microbiol Rev* **16:** 79–95.

Leoni S, Brambilla D, Risuleo G, de Feo G, Scarsella G (2000). Effect of different whole body hyperthermic sessions on the heat shock response in mice liver and brain. *Mol Cell Biochem* **204:** 41–47.

Loiacono CM, Taus NS, Mitchell WJ (2003). The herpes simplex virus type 1 ICP0 promoter is activated by viral reactivation stimuli in trigeminal ganglia neurons of transgenic mice. *J NeuroVirol* **9:** 336–345.

Mackler SA, Homan YX, Korutla L, Conti AC, Blendy JA (2003). The mouse nac1 gene, encoding a cocaine-regulated Bric-a-brac Tramtrac Broad complex/Pox virus and Zinc finger protein, is regulated by AP1. *Neuroscience* **121:** 355–361.

Maroni P, Bendinelli P, Tiberio L, Rovetta F, Piccoletti R, Schiaffonati L (2003). In vivo heat-shock response in the brain: signalling pathway and transcription factor activation. *Brain Res Mol Brain Res* **119:** 90–99.

Morimoto RI (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev* **12:** 3788–3796.

Mulholland NM, Soeth E, Smith CL (2003). Inhibition of MMTV transcription by HDAC inhibitors occurs independent of changes in chromatin remodeling and increased histone acetylation. *Oncogene* **22:** 4807–4818.

Nightingale KP, Wellinger RE, Sogo JM, Becker PB (1998). Histone acetylation facilitates RNA polymerase II transcription of the Drosophila hsp26 gene in chromatin. *EMBO J* **17:** 2865–2876.

Okazaki K, Sagata N (1995). The Mos/MAP kinase pathway stabilizes c-Fos by phosphorylation and augments its transforming activity in NIH 3T3 cells. *EMBO J* **14:** 5048–5059.

Otten U, Marz P, Heese K, Hock C, Kunz D, Rose-John S (2000). Cytokines and neurotrophins interact in normal and diseased states. *Ann N Y Acad Sci* **917:** 322–330.

Perkins D, Pereira EFR, Aurelian L (2003). The herpes simplex virus type 2 R1 protein kinase (ICP10 PK) functions as a dominant regulator of apoptosis in hippocampal neurons involving activation of the ERK survival pathway and upregulation of the antiapoptotic protein Bag-1. *J Virol* **77:** 1292–1305.

Quigney DJ, Gorman AM, Samali A (2003). Heat shock protects PC12 cells against MPP+ toxicity. *Brain Res* **993:** 133–139.

Rizzi N, Denegri M, Chiodi I, Corioni M, Valgardsdottir R, Covianchi F, Riva S, Biamonti G (2004). Transcriptional activation of a constitutive heterochromatic domain of the human genome in response to heat shock. *Mol Biol Cell* **15:** 543–551.

Sakata R, Minami S, Sowa Y, Yoshida M, Tamaki T (2004). Trichostatin A activates the osteopontin gene promoter through AP1 site. *Biochem Biophys Res Commun* **315:** 959–963.

Shaulian E, Karin M (2002). AP-1 as a regulator of cell life and death. *Nat Cell Biol* **4:** E131–E136.

Smith CC, Nelson J, Aurelian L, Gober M, Goswami BB (2000a). Ras-GAP binding and phosphorylation by herpes simplex virus type 2 RR1 PK (ICP10) and activation of the Ras/MEK/MAPK mitogenic pathway are required for timely onset of virus growth. *J Virol* **74:** 10417–10429.

Smith CC, Yu YX, Kulka M, Aurelian L (2000b). A novel human gene similar to the protein kinase (PK) coding domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) codes for a serine-threonine PK and is expressed in melanoma cells. *J Biol Chem* **275:** 25690–25699.

Vaccarino FM, Hayward MD, Le HN, Hartigan DJ, Duman RS, Nestler EJ (1993). Induction of immediate early genes by cyclic AMP in primary cultures of neurons from rat cerebral cortex. *Brain Res Mol Brain Res* **19:** 76–82.

Valyi-Nagy T, Deshmane S, Dillner A, Fraser NW (1991). Induction of cellular transcription factors in trigeminal ganglia of mice by corneal scarification, herpes simplex virus type 1 infection, and explantation of trigeminal ganglia. *J Virol* **65:** 4142–4152.

Wachsman M, Kulka M, Smith CC, Aurelian L (2001). A growth and latency compromised herpes simplex virus type 2 mutant (ICP10-delta-PK) has prophylactic and therapeutic protective activity in guinea pigs. *Vaccine* **19:** 1879–1890.

Wang K, Pesnicak L, Guancial E, Krause PR, Straus SE (2001). The 2.2 kilobase latency-associated transcript of herpes simplex virus type 2 does not modulate viral replication, reactivation, or establishment of latency in transgenic mice. *J Virol* **75:** 8166–8172.

Wymer JP, Chung TD, Chang Y-N, Hayward GS, Aurelian L (1989). Identification of immediate-early-type cis-response elements in the promoter for the ribonucleotide reductase large subunit from herpes simplex virus type 2. *J Virol* **63:** 2773–2784.

Zhu J, Aurelian L (1997). AP-1 cis-response elements are involved in basal expression and vmx110 transactivation of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). *Virology* **231:** 301–312.